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Enhanced Growth and Seed Properties in Introduced vs. Native Populations of Yellow Starthistle (*Centaurea solstitialis*)

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There is much discussion as to why a plant becomes invasive in a new location but is not problematic in its native range. One example is yellow starthistle, which originates in Eurasia and is considered a noxious weed in the United States. We grew yellow starthistle originating from native and introduced regions in a common environment to test whether differences in growth would be observed. In growth chamber studies, seedlings originating from the invasive range were larger than seedlings from the native range after 2 wk. Seed starch content is an important component of initial seedling growth. The starch content of seeds from introduced populations was higher than that of seeds from native populations. Regression analysis showed a relationship between the amount of starch in the seeds and the weight of yellow starthistle seedlings after 2 wk growth. There was no difference in chromosome number, except in accessions originating from Sicily and Sardinia. Field studies conducted in France and Russia revealed that rosettes and mature plants grown under natural conditions were larger when grown from seeds originating from the invasive range than from seeds originating from the native range. The number of capitula per plant and stem diameters were not significant among all populations, but differences were noted. The F1 progeny of plants originating from U.S. seed, but grown and pollinated in France, showed no differences in seedling growth, mature plant characteristics, and seed starch content from the plants grown from field-collected U.S. seed. The changes in seed starch resource allocation and its relation to plant growth is useful in understanding factors that contribute to yellow starthistle's invasibility.

Nomenclature: Yellow starthistle, *Centaurea solstitialis* L. CENSO.

Key words: **C**ompetitive ability hypothesis, invasiveness, invasive species, seeds, starch content.

Movement of plant species, either intentionally or naturally, into new geographical areas has been occurring throughout history. Some of these plants have become invasive and spread well beyond the site of their initial introduction, often displacing native vegetation and disrupting natural ecosystems. In general, there are different theories to explain why plants become invasive and what conditions are involved under which invasions are most likely to occur (Daehler 2003; Grotkopp et al. 2002; Hierro et al. 2005; Maillet and Lopez-Garcia 2000; Rejmánek 1995). A general, but often untested, observation of invasive plants is that they are larger in their introduced ranges than their native ranges. Although Thébaud and Simberloff (2001) found no consistent evidence for increased size among exotics, it has been observed in some studies that plants tend to be more vigorous and taller in invaded environments, producing more seeds than in their native range (Crawley 1987; Jakobs et al. 2004; Willis and Blossey 1999).

Yellow starthistle, family Asteraceae, is considered a noxious weed in the United States. The origins of yellow starthistle are believed to be in Eurasia, where it is not considered a serious weed (Roché and Talbott 1986; Sheley et al. 1999). It is believed that yellow starthistle was introduced into the United States on multiple occasions (DiTomaso 1996; Sun 1997). Plant stand densities have been shown to be significantly lower in its native range than in California, where it is invasive (Uygur et al. 2004). A number of studies have been conducted to explain why yellow starthistle is invasive (Gerlach and Rice 2003; Prather 1994; Roché and Thill 2001; Roché and White 2002). Reasons include the extensive deep-root system, nonoverlapping life history, prolific seed production, and adaptations to an environment with limited water availability. However, many of these factors mentioned above are also present in its native range and, therefore, cannot fully explain its success in its introduced range.

One explanation for differences in plant growth is the Evolution of Increased Competitive Ability (EICA) hypothesis. This hypothesis predicts that invasive plants will allocate more biomass over time to growth and fewer resources to defense in the absence of, or reduced pressure from, specialist herbivory (Blossey and Notzold 1995). The best test for verification of that theory is to study a species collected from both native and invasive habitats and grow them under the same environmental conditions. That would make possible the determination of genetically based changes.

The Enemy Release hypothesis states that a plant species introduced to an exotic region may escape its natural enemies and, thus, increase in distribution and abundance (Keane and Crawley, 2002). Although this is related to the EICA hypothesis, it does not suggest any genetic changes. The escape from natural enemies by yellow starthistle may play a significant role in the success of its invasion into the United States, although no specific study has been conducted to examine this hypothesis. However, in the exhaustive search for biological control agents against this weed, it has been shown that natural enemies of yellow starthistle are more abundant in the native range compared with the introduced range. For example, there are 13 microbial pathogens reported to be associated with this plant in its native range compared with only three in its invasive range (Faggioli et al. 2004; Farr et al. 2006; Klisiewicz 1986; Pitcairn et al. 2000). Although not specifically documented, similar differences between native and introduced ranges are noted with herbivory insects because new, otherwise not reported in North America, insects have been imported into the United States as biological control agents (Piper 2001). If the EICA hypothesis holds true for yellow starthistle, then it makes sense that the biological control agents introduced into the United States from the

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native range should be more effective on this weed if the defense mechanisms have been allocated elsewhere. However, introduced biological control species have had only limited success in managing yellow starthistle, which suggests that (1) the most effective agents have not been found, (2) there has not been enough time for the introduced enemies to have an impact, (3) host-specific natural enemies are not a factor in limiting populations of yellow starthistle, or (4) the EICA hypothesis in relation to specialist natural enemies does not apply to yellow starthistle.

Regardless of whether or not resources have been reallocated from defense into growth, the genetic and phenotypic differences of yellow starthistle in native and introduced ranges have not been explored. The purpose of this study was to determine (1) whether phenotypic differences exist between yellow starthistle from native and introduced regions, and (2) whether yellow starthistle from the two regions are influenced by the environment or by changes in resource allocations. If, under identical environmental conditions, native and introduced populations differ significantly, it would provide evidence for genetic differentiation. We hypothesize that there are differences in growth among introduced and native populations and that these characteristics are influenced by changes in resource allocation that affect growth. Seed starch was examined to study changes in resource allocation because it has been shown to be the primary source of energy used by seedlings during germination. Higher seed starch can increase sugar mobilization to the shoots and roots (Murata et al. 1968). Although this may not be the only factor in seedling establishment, differences in seed starch content between native and introduced populations would show a specific resource-allocation change, implying more than environmental influences.

Materials and Methods

Origin of Plant Material. Mature yellow starthistle achenes (hereafter referred to as seeds) were randomly collected from established populations in July or August from the following locations and years (population codes used throughout this paper are enclosed in brackets): near Montpellier, France (2002 [FR02], 2003 [FR03], 2004 [FR04], and 2005 [FR05]); near Thessaloniki, Greece (2000 [GR00] and 2003 [GR03]); near Adana, Turkey (2001 [TU01] and 2003 [TU03]); near Rome, Italy (2000 [IT00]); near Palermo, Sicily (1999 [SIC99] and 2003 [SIC03]); Sardinia (2000 [SAR00]); near Krasnodar, Russia (2000 [RU00]); and in the United States, near Davis, CA (2000 [CA00]); Putah Creek, CA (2002 [CA02]); near Alameda, CA (2005 [CA05]); near Harriston, ID (2002 [ID02]); and four sites (A, B, C, D) near Hell's Canyon, ID (2005 [ID05A, ID05B, ID05C, and ID05D]). Approximately 10 to 100 plants were sampled at each location with 1 to 10 seed heads collected from each plant. Seeds from an F1 progeny of hand-pollinated flowers from yellow starthistle plants that originated from seeds of CA02 and grown in France in 2004 and 2005 [USFR04 and USFR05] were collected from seven plants with 5 to 10 seed heads per plant. Seeds were stored in paper bags at 4 C until use.

Seedling Growth. To examine the effect of the geographic location of parental plants on seedling growth, the fresh weights of yellow starthistle seedlings were compared between native and introduced ranges. Yellow starthistle produces both pappus- and nonpappus-bearing seed types (Sheley et al. 1999). For trial 1, pappus-bearing seeds of CA05, ID05A, ID05B, ID05C, ID05D, ID02, USFR05, FR05, SIC03, GR03, TU03, and SAR00 were used. For trial 2, pappus-bearing seeds of CA05, ID05A, ID05B, ID05C, ID05D, CA02, USFR05, FR03, TU03, IT00, GR03, RU00, and SIC03 were used. The seeds were germinated on moistened filter paper in a closed plastic petri plate at 25 C. After 1 d, four germinated seeds were transferred to one 200-cm^3 plastic pots containing commercial potting soil.[1] There were five pots per population tested. The pots were placed in a growth chamber at 25 C with a 16-h photoperiod (fluorescent lights) arranged in a completely random manner. Plants were watered on a regular schedule. After 2 wk, the seedlings were carefully removed, and the soil was washed from the roots. Seedlings were then blotted dry and weighed. The fresh weight of the four seedlings in each pot was averaged together for one unit of replication.

Because differences in viability between pappus- and nonpappus-bearing seed have been observed (Maddox et al. 1996), growth differences of seedlings originating from either pappus- or nonpappus-bearing seeds were compared. Seed of each type from ID05A, ID05B, ID05C, ID05D, and FR05 were germinated as described. After 1 d, five germinated seeds were transferred to one 200-cm^3 plastic pots containing commercial potting soil[1] and grown as described. There were four pots of five seedlings per pot from each location. After 2 wk, data from these seedlings were collected as described. The experiment was repeated once.

Seed-Starch Analysis. Seed populations from the United States (CA02, CA05, ID02, ID05A, ID05B, ID05C, ID05D), France (FR03, FR05, USFR05), Italy (IT00), Sicily (SIC03), Greece (GR03), Russia (RU00), and Turkey (TU03) were tested for starch determination. Pappus- and nonpappus-bearing seeds were separated. Average individual seed weight was determined by weighing 100 pappus-bearing seeds, drying them in an oven at 80 C for 48 h, and weighing them again. This was repeated three times for each seed origin tested.

Determination of starch in yellow starthistle seeds was conducted following the methods described in the Sigma Starch Assay Kit, STA-20.[2] For each population of seed, four replicates of seed, weighing between 50 and 150 mg each, were weighed and transferred to a test tube. Briefly, dehydrated seeds (80 C for 48 h) were pretreated with 80% ethanol to remove any glucose or maltodextrins. After centrifugation, the resulting pellet was subjected to starch digestion by adding 0.2 ml of 80% ethanol to each sample and to a blank tube, mixing, and adding 3.0 ml of deionized water and 0.02 ml of the α-amylase reagent provided by Sigma. The tubes were mixed, incubated for 5 min in a boiling-water bath, and brought up to a volume of 10 ml with deionized water after cooling to room temperature. To 1.0 ml of each sample solution, 1.0 ml of the starch assay reagent provided by Sigma was added, mixed, and incubated for 15 min in a 60 C water bath. The tubes were allowed to cool to room temperature, and 1.0 ml from each test and blank sample was diluted to 10 ml with deionized water.

To determine the amount of glucose in the samples, 1.0 ml of the sample from the starch digestion was mixed with 2.0 ml of the provided Sigma glucose assay reagent. For the standard and reagent blanks, instead of the sample solution, 1.0 ml of water or 1.0 ml of the blank solution after starch digestion was used, respectively. A glucose standard was prepared by mixing 0.95 ml of deionized water and 0.05 ml of the provided Sigma standard solution. Tubes were incubated for exactly 30 min at 37 C. The reaction was stopped by adding 2.0 ml of 12 N sulfuric acid into each tube and mixing thoroughly. The absorbance of each tube was measured at 540 nm. The percentage of global starch was calculated by the formula provided by Sigma:

$$\left[\left(A_{test} - A_{reagent\ blank}\right) \times 9{,}000\right] / \left[\left(A_{standard} - ABS_{standard\ blank}\right)(\text{mg sample weight})\right] \quad [1]$$

The weight of starch per seed was calculated by multiplying the average weight of seed by the percentage of starch per seed. The percentage and weight (μg) of starch per seed were calculated for each population.

Plant Growth. In three separate field experiments, yellow starthistle from different populations were grown to maturity at two locations. At location 1 (Montferrier sur Lez, France) in October, 2003, yellow starthistle seeds CA02, FR03, IT00, SIC99, GR00, and TU01 were germinated as described, and seedlings were transplanted to 200-cm^3 plastic pots containing commercial potting soil.[1] The pots were placed in a greenhouse in a completely random manner under natural lighting and watered on a regular schedule. In December of the same year, ≥ 10 seedlings from each geographical location were transplanted to separate 1-m^2 plots consisting of a natural clay soil (clay 79.6% : silt 11.5% : sand 8.9%) and received only natural rainfall. The arrangement of the plots was blocked in a completely randomized design. In April of the following year, the rosette diameter of each surviving yellow starthistle plant was measured. Approximately 20 immature capitula from each plant were bagged with a fine-mesh screen in the end of May, and in June of that same year, flowers of yellow starthistle from U.S. populations were hand-pollinated with flowers of different yellow starthistle plants also originating from the same U.S. population. These flowers were rebagged to prevent bees from pollinating them and to contain the seeds. Mature seeds were harvested and stored in a paper bag at 4 C for further use. In July, plant height, number of capitula, and stem diameter were measured for each plant. The area occupied by the plant was calculated by measuring the plant diameter in two perpendicular directions with the intersection at the center of the plant. The longest diameter was chosen as one of the directions. These two measurements were multiplied by the plant height to give the estimated plant canopy. In October 2004, the experiment was repeated as previously described, except the field plot included seeds from a Russian population (RU00) and the F1 progeny of U.S. plants produced in the field the previous season.

At location 2, near Krasnodar, Russia, in April 2005, yellow starthistle seeds of CA02, USFR04, FR04, IT99, SIC99, GR03, RU00, and TU01 were planted directly into field plots in a loam soil (clay 24.7% : silt 32.6% : sand 42.7%). In May 2005, the rosette diameter of each surviving yellow starthistle plant was measured. At the end of July of that same year, plant measurements were taken as previously described for location 1.

To prevent the risk of introducing new genotypes, field sites were chosen that were isolated from any known established yellow starthistle populations (minimum 30 km). In addition, immature capitula not used for crossing experiments were removed before seed set, and field sites were monitored the following year for seedlings. Those found were removed.

Chromosome Counts. Chromosome counts were conducted with a modified protocol by D'Hont et al. (2000). Root tips were removed from germinated seeds of U.S. (CA00), French (FR02), Italian (IT00), Sicilian (SIC99), Sardinian (SAR00), Greek (GR00), Turkish (TU01), and Russian (RU00) populations, infiltrated for 15 min with 0.04% 8-hydroxyquinoline[3] at room temperature and then allowed to sit at 4 C for an additional 4 h. Samples were fixed in ethanol and glacial acetic acid (3 : 1 by vol) overnight at 4 C and prepared for digestion by washing them in distilled water for 10 min, 0.25 N HCl for 10 min, and again in distilled water for 10 min. The root was excised from each seedling and placed in an enzymatic solution (5% Onozuka R10 cellulase,[4] 1% Pectolyase Y23[5]) for 30 min in a 37 C water bath. Root tips were removed, placed in distilled water for 30 min, and transferred to a glass slide. The excess water was removed from each specimen and one or two drops of the ethanol and glacial acetic acid fixative solution were added. The root tips were stained with a 1 μl ml^{-1} DAPI stock solution (1 mg ml^{-1} 4′,6-diamidino-2 phenylindole dihydrochloride[6] in McIlvaine Buffer, pH 7.0 [18.2 ml of 0.1 M citric acid and 81.8 mL of 0.2 M Na_2HPO_4]) in Vectashild solvent.[7] Chromosomes were observed microscopically[8] with fluorescence illumination (excitation: 340 to 380 nm, suppression: 430 nm).

Statistical Analyses. All statistical analyses were conducted using SAS for Windows[9] (Version 8.02) except where noted. Differences of seedling fresh weights between populations were examined by subjecting the data to ANOVA. Each trial was analyzed separately because variances were not equal. The data were transformed by square root before analysis to equalize the variance within trials (Gomez and Gomez 1984). Data are presented as nontransformed means. Means were separated using Fisher's Protected LSD at the P = 0.05 confidence level.

Fresh weights of 2-wk-old seedlings grown from pappus- or nonpappus-bearing seeds were analyzed statistically with two-factor ANOVA to test for differences between populations and seed type. Data from the repeated trials in each experiment were combined because results were not statistically different based on an *F* test. Means were separated using Fisher's Protected LSD at the P = 0.05 confidence level.

Percentage of global starch and weight of starch per seed for each repetition were transformed using the arcsine square root and log(*x*), respectively, to stabilize variance (Gomez and Gomez 1984) before ANOVA. Transformed means were separated using a Fisher's Protected LSD at P = 0.05. A two-factor ANOVA was performed to test statistical significance of population and seed type. Nontransformed means are presented in the tables for clarity. Dry seed weight data did not require transformation before analysis. Means were separated for dry seed weight data as previously described.

Table 1. Yellow starthistle seedling weight and pappus-bearing seed starch analyses from different geographical locations.

Location[b]	Plant weight[a]		Seed weight	Starch[c]	Starch[d]
	Trial 1	Trial 2			
	mg			%	μg
Idaho	130 a[e]	64 a	1.72 a	2.30 a	35.3 abc
California	124 ab	56 a	1.79 a	2.62 a	47.1 a
U.S. progeny (F1)	122 ab	57 a	1.91 a	2.33 a	41.1 ab
Turkey	114 b	56 a	1.71 a	1.51 bc	25.0 cd
Italy	—	41 b	0.85 c	1.29 c	10.4 d
Greece	80 c	38 b	0.90 c	1.55 bc	16.1 d
France	72 c	40 b	1.01 bc	1.43 bc	13.1 d
Russia	—	37 b	1.01 bc	2.03 ab	20.5 cd
Sicily	48 d	34 b	1.28 b	1.46 bc	17.0 d
Sardinia	44 d	—	—	—	—

[a] Average plant weight (mg) of yellow starthistle seedlings after 2 wk growth.
[b] Geographic location where seeds were collected. U.S. progeny (F1) were seeds produced by plants originating from U.S. seeds and grown and pollinated in France. Data from locations with more than one accession were pooled for that location.
[c] Average percentage of starch in a seed of yellow starthistle as determined spectrophotometrically.
[d] Average amount (μg) of starch per seed of yellow starthistle.
[e] Values followed by the same letter are not significantly different ($P < 0.05$).

Both seed weight and the weight of starch per seed were compared with the weight of the 2-wk-old seedlings using regression analysis (SigmaPlot,[10] Version 6.00); starch content and seed weight were the independent variables.

For the field-plot samples of each trial location and trial year, data for rosette diameter, plant height, number of capitula, stem diameter, and plant canopy (the dependent variables) were square root transformed. Nested ANOVA, using PROC GLM, was used to analyze the variation in rosette diameter, plant height, canopy volume, stem diameter, and the number of capitula per plant among seed origins, field-site locations, and site and origin interaction with the trial year nested within the seed origin as the error term. Means of the dependent variables were separated by the seed origin using Fisher's Protected LSD at the $P = 0.05$ level confidence.

Results and Discussion

It is estimated that there are thousands of seeds from exotic species accidentally introduced into new regions each year (Crawley 1986). However, only a small fraction of these introduced species become invasive (Williamson 1993). Once a plant is introduced and established, it must have certain characteristics that enable it to outcompete native or nonnative species and spread as an invasive. Although not measured in this study, root growth is known to be one of the most important characteristics of yellow starthistle that enables it to be an effective invader (Gerlach et al. 1998). Deep root growth extends the period of resource availability into late summer, greatly benefiting yellow starthistle in the dry summer months typical of a Mediterranean and western U.S. climate (Enloe et al. 2004; Sheley et al. 1993). It has been demonstrated that plants that rapidly develop tall and leafy shoots have a competitive advantage over slower growing species (Bozsa and Oliver 1990; Gonzalez Ponce et al. 1996). In addition, healthy and vigorous seedlings generally are more resistant to natural enemies, thus increasing their survival and performance. Shading of young yellow starthistle seedlings and rosettes can have a dramatic effect on root growth (DiTomaso et al. 2003; Roché et al. 1994). Consequently, yellow starthistle does not survive well in shaded areas and is less competitive in areas dominated by shrubs, trees, tall perennials, and grasses. Thus, it would be advantageous for yellow starthistle seedlings to grow quickly, enabling them to compete for ample sunlight and establish a deep root system.

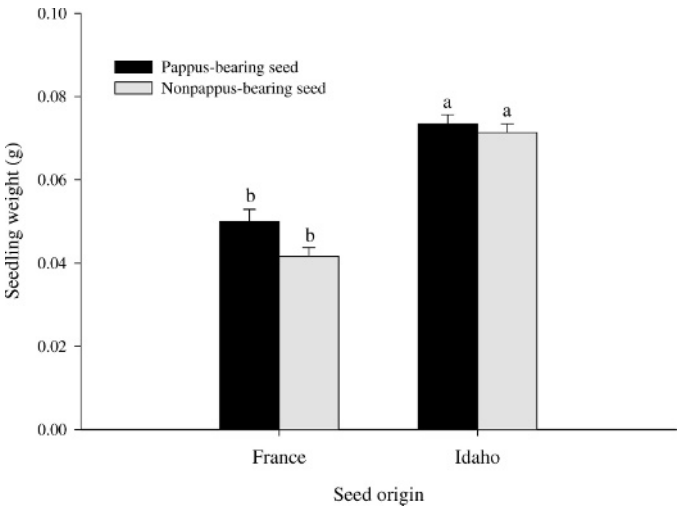

Figure 1. Comparison of yellow starthistle seedling weight grown from pappus- and nonpappus-bearing seeds originating from France or Idaho. Columns with the same letter are not significantly different using Fisher's Protected LSD test at $P = 0.05$. Bars represent the standard error of the means.

Seedling Growth. Yellow starthistle seeds from all geographical locations germinated on the filter paper within 24 h. After 2 wk in the growth chamber, there was a significant difference in seedling weight between the two trials, so the data were not combined ($P < 0.01$). Overall, the average fresh weight of yellow starthistle seedlings after 2 wk from the invaded range (120.3 ± 3.1 mg in trial 1; 61.1 ± 0.9 mg in trial 2) was greater ($P < 0.001$) than the average fresh weight of seedlings grown from seeds originating from the native range (76.5 ± 4.2 mg in trial 1; 41.8 ± 1.3 mg in trial 2). Considered according to geographical location, populations from Turkey were not significantly different in seedling weight from some of the seedlings originating from the United States ($P < 0.001$; Table 1). Seedling weight was not affected by type of seed (pappus- or nonpappus-bearing) within location (Figure 1). Differences in seedling growth

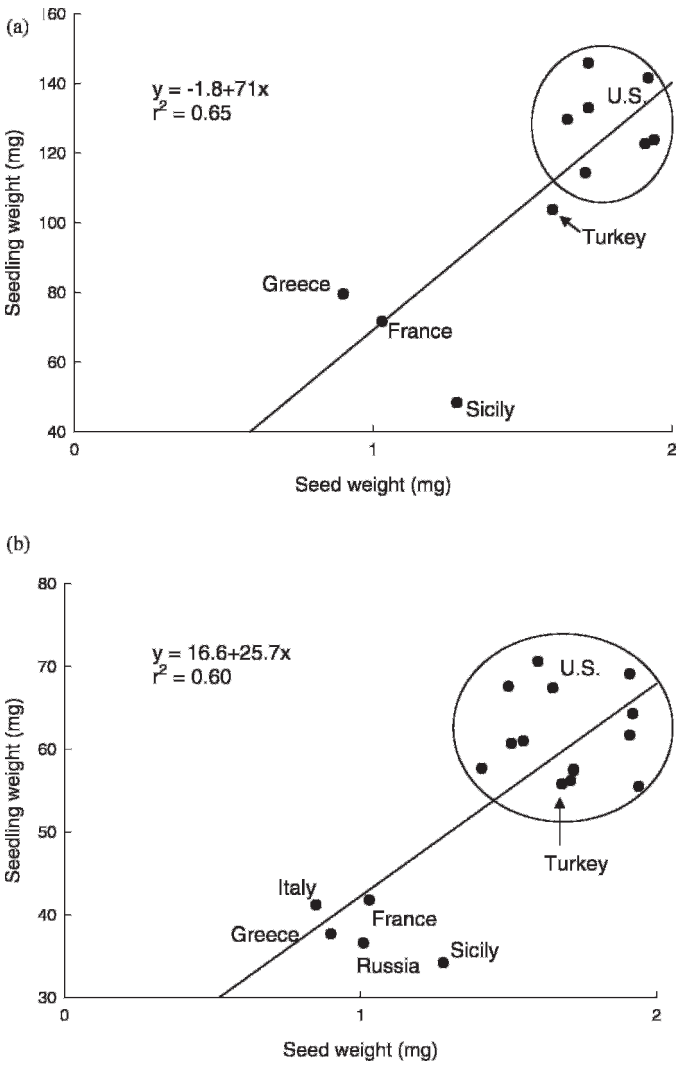

Figure 2. Linear regression analysis of the relationship between seed and seedling weight of yellow starthistle after 2 wk for (a) trial 1 and (b) trial 2. Country name beside data point clarify location for each data point, and data points encompassed in circle are from U.S. populations.

observed in this study may be one factor in explaining invasiveness of U.S. populations.

Seed-Starch Analysis. Results of this study clearly showed a relationship between seed weight and seedling weight. The mean seed weight from U.S. populations was greater than those of native populations, except for the Turkish population (Table 1). Nelson et al. (1970) observed a positive correlation between seed weight and seedling vigor in the case of medusahead rye [*Taeniatherum asperum* (Simonk.) Nevski (= *Taeniatherum caput-medusae* (L.) Nevski]. Many constituents within the seed can affect seed size. This can be influenced by genetics, the environment, or available resources. In general, seeds of the Asteraceae contain an endosperm of two to three cell layers just below the pericarp. The primary constituent of the endosperm is starch, and starch grains vary in size between accessions. Greater relative energy supply in seed of an individual or a population would enable more rapid growth, reducing the probability of effect of shading from other plants. In this study, the percentage of seed starch measured spectrophotometrically differed significantly among populations ($P = 0.031$; Table 1). U.S. seeds contained the highest percentage of starch, not significantly different from seeds of the Russian population. Similarly, the amount of starch per seed was significantly different ($P = 0.003$), depending upon the population (Table 1). There was no difference in starch content between pappus- and nonpappus-bearing seeds within populations (data not shown).

Despite the unexplained difference in seedling weight between the two trials, the relationship between seed and seedling weight was linear. Regression analysis showed a linear relationship between seed weight and seedling growth in both trial 1 ($P = 0.003$; $R^2 = 0.65$; Figure 2A) and trial 2 ($P < 0.001$; $R^2 = 0.60$; Figure 2B). There was also a linear relationship between the percentage of starch per seed and seedling weight for both trial 1 ($P = 0.0012$; $R^2 = 0.52$; data not shown) and trial 2 ($P < 0.001$; $R^2 = 0.49$; data not shown). The higher percentage of starch in seeds from invasive habitats presented in this study could be an important factor to explain specifically why yellow starthistle is invasive in the United States and suggests that resource allocation has changed.

Chromosome Analysis. The number of chromosomes of yellow starthistle from different populations was compared as a possible explanation for the increase in growth of U.S. seedlings. Chromosome counts of yellow starthistle from the United States, France, Greece, Russia, Turkey, and Italy were $2N = 16$, which agrees with the results of an earlier study by Love (1981). Chromosome counts of populations from Sicily and Sardinia were $2N = 18$ and, thus, may support description of a yellow starthistle subspecies, *Centaurea solstitialis* subsp. *schouwii* (DC.) Dostal, that is endemic to Sicily and Sardinia (Dostál 1976; Wagenitz 1975). Morphological verification of these accessions was not conducted in this study. Seedling weights from these populations were lower than the others in one of the two trials (Table 1). Thus, it does not appear that the extra chromosome has a positive effect on seedling growth or weight.

Field Plot. Differences in growth characteristics of mature yellow starthistle plants were similar to those for seedlings. Seed origin significantly influenced rosette diameter, canopy volume of mature plants, plant height, and stem diameter ($P < 0.05$; Table 2). There was a difference in the field site on rosette diameter, stem diameter, and the number of capitula. Interaction between the field site and the seed origin also was observed for these dependent variables. Variation due to the trial year nested within the origin of the seed was only observed in the number of capitula and plant height. Rosette diameters of both U.S. and U.S. progeny (F1) plants were larger than those originating from native populations (Figure 3). Canopy volume was also larger except at the Russian field site where the canopy volumes of the French plants were not significantly different from the U.S. or U.S. progeny (F1). Plants of F1 progenies of selfed U.S. accessions were not different from the parental genotypes of U.S. populations. Yellow starthistle seeds originating from Russia at location 2 either did not germinate or did not survive past the seedling stage, so data could not be recorded.

Table 2. Summary of nested analysis for rosette diameter, canopy volume, stem diameter, and number of capitula in yellow starthistle populations originating from different geographical areas and grown in two separate locations in 2004 and 2005. Trial year (seed origin) was used as the error term.

Dependent variable	Source of Variation	df	*F* value	P value
Rosette diameter	Seed origin	7	16.50	0.0035
	Trial year (seed origin)	5	0.83	0.5285
	Field site × seed origin	6	2.71	0.0154
	Field site	1	104.67	< 0.0001
	Trial year	1	6.23	0.0135
Plant height	Seed origin	7	5.25	0.0432
	Trial year (seed origin)	5	2.98	0.0131
	Field site × seed origin	6	0.47	0.8296
	Field site	1	0.02	0.8532
	Trial year	1	7.83	0.0057
Canopy volume	Seed origin	7	5.86	0.0346
	Trial year (seed origin)	5	1.57	0.1715
	Field site × seed origin	6	1.81	0.0998
	Field site	1	3.81	0.0525
	Trial year	1	1.12	0.2914
Stem diameter	Seed origin	7	36.21	0.0005
	Trial year (seed origin)	5	0.24	0.9442
	Field site × seed origin	6	4.12	0.0007
	Field site	1	18.49	< 0.0001
	Trial year	1	59.79	< 0.0001
Number of capitula	Seed origin	7	0.20	0.9723
	Trial year (seed origin)	5	4.30	0.0010
	Field site × seed origin	6	3.05	0.0074
	Field site	1	48.97	< 0.0001
	Trial year	1	16.29	< 0.0001

Results in this study show several differences in characteristics between plants originating from invaded and native ranges grown in a common environment. Thus, this data would not support the Environmental Constraint hypothesis as a reason for yellow starthistle invasiveness, which states limited resource availability to the plant in its native range is the main factor determining this discrepancy (Bryant et al. 1988; Galatowitsch et al. 1999). If the constraints are removed then plants can invade habitats from which they were restricted. For example, Rickey and Anderson (2004) found that common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] benefits from increased soil nitrogen and is more likely to displace a native grass in a nitrogen-rich environment. Burns (2004) found similar results in that invasive species had higher relative growth rates than noninvasive congeners at high nutrient availabilities but did not differ at low nutrient availabilities. Besides growth, environmental factors have been shown to affect other plant characteristics, such as fecundity, that can influence invasiveness (Edwards et al. 1998).

The EICA hypothesis proposes that growth changes and invasiveness are driven by escaping specialist enemies, where resources are allocated from defense to growth (Blossey and Notzold 1995). In this study, larger plants and higher seed starch content observed in specimens originating from an invasive range suggests increased resource allocation toward growth. However, it cannot be stated where these resources were allocated from because biodefence mechanisms were not directly examined in this study. In addition, no distinct differences were observed in the number of capitula, and therefore, it cannot be concluded that resources are allocated to both an increase in vegetative growth and reproduction. Counting the number and viability of seeds, which was not determined in this study, would be a true test of increase reproduction ability. To elucidate whether resources are being allocated toward growth and taken from defense mechanisms, further studies need to be done to compare the susceptibility of seedlings or plants originating from native habitats and from the United States or other invaded locations to a variety of biological control agents.

Several studies demonstrated that plants grown from seed originating from invaded ranges were larger than plants originating from the native range (Blossey and Notzold 1995; Siemann and Rogers 2001). Edwards et al. (1998) had similar results but indicated that this difference was affected by soil quality and nutrient availability. However, Willis et al. (2000) found no growth differences in a common environment growth experiment of musk thistle (*Carduus nutans* L.), foxglove [*Digitalis purpurea* L.], tansy ragwort (*Senecio jacobaea* L.), and blueweed (*Echium vulgare* L.). Bossdorf et al. (2005) provide a review of all currently available data on comparisons of native vs. introduced plant populations. They conclude that plant characteristics (i.e., size, fecundity) that are used to explain invasiveness of a plant may depend upon the specific scenario of plant and environment and that generalizations should be made with caution. The result of removing various selection pressures in a new environment, such as enemy escape, can be rapid genetic change (Hanfling and Kollman 2002; Lee 2002; Sakai et al. 2001). However, not all data from the various studies previously conducted comparing plant growth of invasive species to native species conclusively support the EICA hypothesis (Colautti et al. 2004). Several studies have demonstrated that there are differences in plant response to specialist and generalist herbivore attack, which may explain the discrepancies in conclusions from the different studies (Joshi and Vrieling 2005; van der Meijden 1996).

It is important not to attribute all of these growth differences to a genetic change in resource allocation. Many studies show that maternal effects are responsible for growth and defense differences (Agrawal 2001; Buckley et al. 2003; Galloway 2005; Heppell et al. 1998). Maternal effects (i.e.,

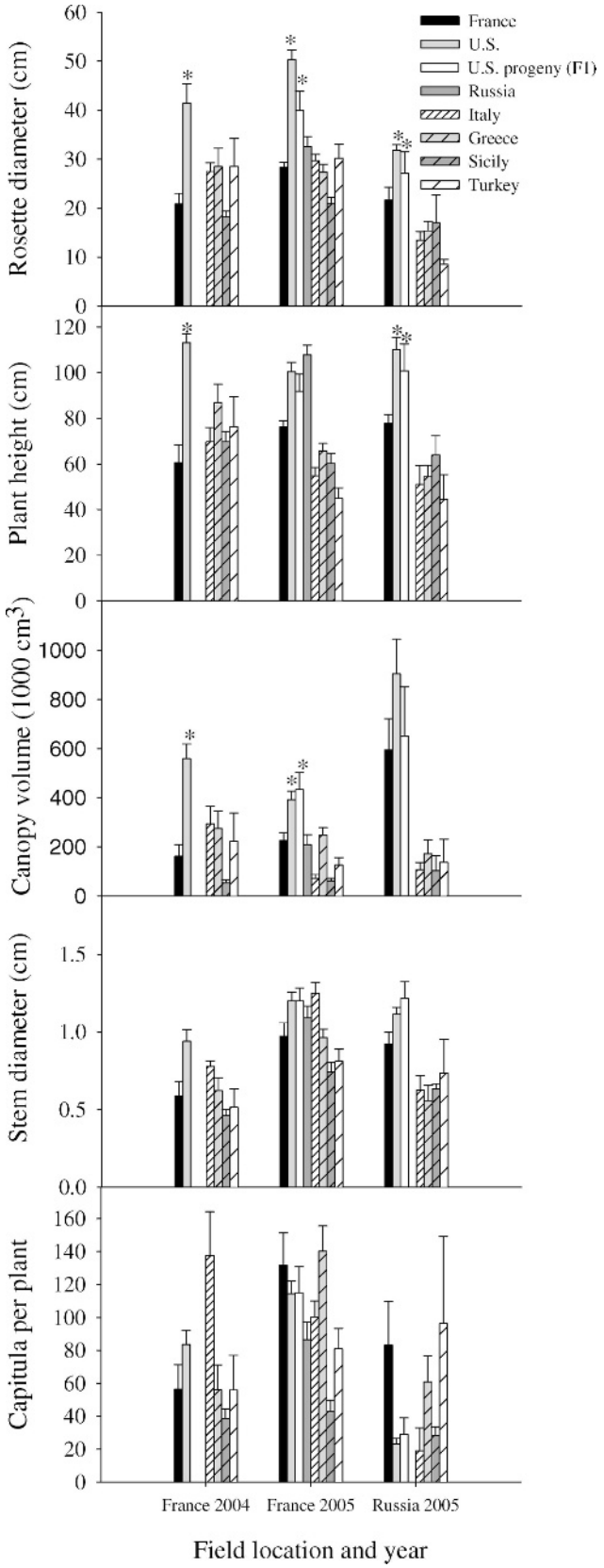

Figure 3. Comparison of seed origin on rosette diameter, plant height, canopy volume, stem diameter, and the number of capitula per plant of yellow starthistle grown either in France or Russia in 2004 and 2005. Bars represent the standard error of the means; asterisks (*) indicate significantly different comparisons within a similar field-plot location and year to seed origins from native locations ($P < 0.05$).

nongenetic differences in seed quality) are a form of phenotypic plasticity that occurs across generations (Agrawal 2002). In other words, some external factor (e.g., herbivory, environmental) causes activation of genes that, when expressed, are manifest as different phenotypes. The results in such differences in performance occur particularly in the earliest stages of growth (Agrawal 2002). In this experiment, early growth differences were observed, and those differences were maintained to plant maturity. Although we cannot exclude the possibility, we do not believe that maternal effects explain our differences. Seed size has been used as an indicator of maternal effects in other studies, with larger seed sizes relating to seedling fitness (Agrawal 2001; Nelson et al. 1970). These effects may persist and affect the flowering and fruiting in mature plants as well (Stanton 1984; Wulf 1986). In this study, seeds originating from the United States were, in general, larger than seeds from native populations, except from Turkey. Despite the fact that the Turkish seeds were the same size as the U.S. seeds, they had a significantly lower percentage of starch, with levels comparable to other native population seeds. Also, rosettes and mature plants grown from the Turkish population in the garden experiment were smaller than those of U.S. accessions even though the 2-wk-old seedlings were similar in weight. In addition, the F1 progeny of U.S. seeds grown and pollinated in the native range expressed the same qualities (larger plant growth and higher seed starch content) as the parents, when grown under the same conditions. By itself, the fact that the F1 progeny were the same size is not enough proof that the characteristics were genetically transferred because maternal effects have been reported to persist to the third generation in other plant species (Miao et al. 1991; Wulf et al. 1999). Additional generations would need to be produced and tested to verify that this is a genetic transfer. However, the F1 progeny data suggest that the environment did not reverse the effect of these characteristics.

Based upon regression analysis of starch weight per seed vs. weight of 2-wk-old seedlings, the closest relative to the U.S. population appears to be the Turkish population. Although this is not conclusive proof that yellow starthistle in the United States originated from Turkey, it does support the finding of Berner and Paxson (2003). They found that reactions of yellow starthistle from California to infection by the rust *Puccinia jaceae* were not different from those of Turkish populations. It is believed that a host-specific pathogen will react differently to different genotypes that did not coevolve. However, if genetic resources have been allocated away from defense to growth then different reactions to pathogens would also be expected. Genetic analyses currently being conducted will give insights that are more definitive as to the origin of U.S. populations (S. J. Anderson, personal communication).

An understanding of the biology of yellow starthistle and its growth is important in better explaining why this species is a severe problem in the United States and not in its native habitat. The larger reserve of starch in the U.S. seed likely provides increased energy necessary for seedling growth. This increase in seedling growth could give these plants an early competitive advantage against other plants. This does not fully explain why yellow starthistle is such a problem in the United States. For example, U.S. populations also lack the full compliment of native predators and pathogens. In addition, the effect of native vegetation, environmental

adaptability, or other physical properties may contribute to the invasibility of yellow starthistle in the United States. Including more geographically isolated populations from other invaded sites outside the United States, such as South Africa, would provide additional insight on the invasive mechanism of yellow starthistle in the United States. Results from that study may be very different, however, depending on the time of introduction and the number of introductions for potential hybridizations. Overall, understanding these different factors of invasiveness will give better insight into what management options will be the most effective.

Sources of Materials

[1] Humin substrat N2, Neuhaus, Klasmann-Deilmann GmbH, Geeste-Groβ Hesepe, The Netherlands.

[2] Starch Assay Kit STA-20, Sigma-Aldrich Chimie S.A.R.L., L'Isle d'Abeau Chesnes, B.P. 701, 38297 Saint Quentin Fallavier, France.

[3] 8-hydroxyquinolein, Fluka Chemie GmbH, Gruenwalder Weg 30, 82041 Deisenhofen, Germany.

[4] Onozuka R10 cellulase, Yakult Pharmaceutical Co., 13-5 Shinbashi 5-Chome, Minatoku, Tokyo 105-0004 Japan.

[5] Pectolyase Y23, Sheishin Pharmaceutical Co., Tokyo, Japan.

[6] 4′,6-diamidino-2 phenylindole dihydrochloride, Sigma-Aldrich Chimie S.A.R.L., L'Isle d'Abeau Chesnes, B.P. 701, 38297 Saint Quentin Fallavier, France.

[7] Vectashild solvent, Vector Lab., 30 Ingold Road, Burlingame, CA 94010.

[8] Microscope Model DMR XA, Leica Microsystems AG, Ernst-Leitz-Strasse 17-37, 35578 Wetzlar, Germany.

[9] SAS v. 8.02, SAS Institute, Inc., SAS Campus Drive, Cary, NC 27513.

[10] SigmaPlot v. 6.00, SPSS Inc., 233 S. Wacker Drive, Chicago, IL 60606.

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Literature Cited

Agrawal, A. A. 2001. Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? Am. Nat. 157:555–569.

Agrawal, A. A. 2002. Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. Ecology 83:3408–3415.

Berner, D. K. and L. K. Paxson. 2003. Use of digital images to differentiate reactions of collections of yellow starthistle (*Centaurea solstitialis*) to infection by *Puccinia jaceae*. Biol. Control 28:171–179.

Blossey, B. and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J. Ecol. 83:887–889.

Bossdorf, O., H. Auge, L. Lafuma, W. E. Rogers, E. Siemann, and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11.

Bozsa, R. C. and L. R. Oliver. 1990. Competitive mechanisms of common cocklebur (*Xanthium strumarium*) and soybean (*Glycine max*) during seedling growth. Weed Sci. 38:344–350.

Buckley, Y. M., P. Downey, S. V. Fowler, R. Hill, J. Memmott, H. Norambuena, M. Pitcairn, R. Shaw, A. W. Sheppard, C. Winks, R. Wittenberg, and M. Rees. 2003. Global patterns of seed size variation in invasive plants. Ecology 84:1434–1440.

Burns, J. H. 2004. A comparison of invasive and noninvasive dayflowers (Commelinaceae) across experimental nutrient and water gradients. Divers. Distrib. 10:387–397.

Bryant, J. P., J. Tuomi, and P. Niemela. 1988. Environmental constraint of constitutive and long-term inducible defenses in woody plants. Pages 367–389 *in* K. C. Spencer, ed. Chemical Mediation of Coevolution. San Diego, CA: Academic.

Colautti, R. I., A. Ricciardi, I. A. Grigorovich, and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? Ecol. Lett. 7:721–733.

Crawley, M. J. 1987. What makes a community invasible? Pages 429–453 *in* A. J. Gray, M. J. Crawley, and P. J. Edwards, eds. Colonization, Succession and Stability. Oxford: Blackwell Scientific.

Daehler, C. C. 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annu. Rev. Ecol. Evol. Syst. 34:183–211.

D'Hont, A., A. Paget-Goy, J. Escoute, and F. Carreel. 2000. The interspecific genome of cultivated banana, *Musa* spp. revealed by DNA *in situ* hybridisation. Theor. Appl. Genet. 100:177–183.

DiTomaso, J. M. 1996. Yellow starthistle: biology and life history. Pages 61–64 *in* J. Lovich, J. Randall, and M. Kelly, eds. Proceedings of the California Exotic Pest Plant Council Symposium, Volume 2. Sacramento, CA: California Exotic Pest Plant Council.

DiTomaso, J. M., G. B. Kyser, and C. B. Pirosko. 2003. Effect of light and density on yellow starthistle (*Centaurea solstitialis*) root growth and moisture use. Weed Sci. 51:334–341.

Dostál, J. 1976. *Centaurea* L. Pages 254–301 *in* T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb, eds. Flora Europea, Volume 4. Cambridge: Cambridge University Press.

Edwards, K. R., M. S. Adams, and J. Kvet. 1998. Differences between European native and American invasive populations of *Lythrum salicaria*. J. Veg. Sci. 9:267–280.

Enloe, S. F., J. M. DiTomaso, S. B. Orloff, and D. J. Drake. 2004. Soil water dynamics differ among rangeland plant communities dominated by yellow starthistle (*Centaurea solstitialis*), annual grasses, or perennial grasses. Weed Sci. 52:929–935.

Faggioli, F., G. Pasquini, V. Lumia, G. Campobasso, T. L. Widmer, and P. C. Quimby, Jr. 2004. Molecular identification of a new member of the clover proliferation phytoplasma group (16SrVI) associated with Centaurea solstitialis virescence in Italy. Eur. J. Plant Pathol. 110:353–360.

Farr, D. F., A. Y. Rossman, M. E. Palm, and E. B. McRay. 2006. Fungal Databases: Systemic Botany and Mycology Laboratory. http://nt.ars-grin.gov/fungaldatabases/. Accessed: January 21, 2006.

Galatowitsch, S. M., N. O. Anderson, and P. D. Ascher. 1999. Invasiveness in wetland plants in temperate North America. Wetlands 19:733–755.

Galloway, L. F. 2005. Maternal effects provide phenotypic adaptation to local environmental conditions. New Phytol. 166:93–100.

Gerlach, J. D., A. Dyer, and K. J. Rice. 1998. Grassland and foothill woodland ecosystems of the Central Valley. Fremontia 26:39–43.

Gerlach, Jr., J. D. and K. J. Rice. 2003. Testing life history correlates of invasiveness using congeneric plant species. Ecol. Appl. 13:167–179.

Gomez, K. A. and A. A. Gomez. 1984. Test for homogeneity of variance. Pages 467–471 *in* K. A. Gomez and A. A. Gomez, eds. Statistical Procedures for Agricultural Research. 2nd ed. New York: J. Wiley.

Gonzalez Ponce, R., C. Zancada, M. Verdugo, and L. Salas. 1996. Plant height as a factor in competition between black nightshade and two horticultural crops (tomato and pepper). J. Hortic. Sci. 71:453–460.

Grotkopp, E., M. Rejmánek, and T. L. Rost. 2002. Toward a causal explanation of plant invasiveness: seedling growth and life-history strategies of 29 pine (*Pinus*) species. Am. Nat. 159:396–419.

Hanfling, B. and J. Kollman. 2002. An evolutionary perspective on invasions. Trends Ecol. Evol. 17:545–546.

Heppell, K. B., D. L. Shumway, and R. T. Koide. 1998. The effect of mycorrhizal infection *Abutilon theophrasti* on competitiveness of offspring. Funct. Ecol. 12:171–175.

Hierro, J. L., J. L. Maron, and R. M. Callaway. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. J. Ecol. 93:5–15.
Jakobs, G., E. Weber, and P. J. Edwards. 2004. Introduced plants of the invasive *Solidago gigantean* (Asteraceae) are larger and grow denser than conspecifics in the native range. Divers. Distrib. 10:11–19.
Joshi, J. and K. Vrieling. 2005. The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. Ecol. Lett. 8:704–714.
Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17:164–170.
Klisiewicz, J. M. 1986. Susceptibility of yellow starthistle to selected plant pathogens. Plant Dis. 70:295–297.
Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17:386–391.
Love, A. 1981. Chromosome number reports LXXIII. Taxon 30:829–861.
Maddox, D. M., D. B. Joley, D. M. Supkoff, and A. Mayfield. 1996. Pollination biology of yellow starthistle (*Centaurea solstitialis*) in California. Can. J. Bot. 74:262–267.
Maillet, J. and C. Lopez-Garcia. 2000. What criteria are relevant for predicting the invasive capacity of a new agricultural weed? The case of invasive American species in France. Weed Res. 40:11–26.
Miao, S. L., F. A. Bazzaz, and R. B. Primack. 1991. Persistence of maternal nutrient effects in *Plantago major*: the third generation. Ecology 72:1634–1642.
Murata, T., T. Akazawa, and S. Fukuchi. 1968. Enzymatic mechanism of starch breakdown in germinating rice seeds, I: an analytical study. Plant Physiol. 43:1899–1905.
Nelson, J. R., G. A. Harris, and C. J. Goebel. 1970. Genetic vs environmentally induced variation in medusahead [*Taeniatherum asperum* (Simonkai) Nevski]. Ecology 51:526–529.
Piper, G. L. 2001. The biological control of yellow starthistle in the western United States: four decades of progress. Pages 48–55 *in* L. Smith, ed. Proceedings of the First International Knapweed Symposium of the Twenty-First Century. Albany, CA: U.S. Department of Agriculture–Agricultural Research Service.
Pitcairn, M. J., D. M. Woods, D. B. Joley, D. G. Fogle, and V. Popescu. 2000. Impact of seedling pathogens on yellow starthistle in California. Pages 52–54 *in* D. M. Woods, ed. Biological Control Program Annual Summary, 1999. Sacramento, CA: California Department of Food and Agriculture, Plant Health and Pest Prevention Services.
Prather, T. S. 1994. Biology of yellow starthistle. Pages 219–223 *in* Proceedings of the California Weed Conference. Volume 46. Fremont, CA: California Weed Science Society.
Rejmánek, M. 1995. What makes a species invasive? Pages 3–13 *in* P. Pysek, K. Prach, M. Rejmánek, and M. Wade, eds. Plant Invasions: General Aspects and Special Problems. Amsterdam: SPB Academic.
Rickey, M. A. and R. C. Anderson. 2004. Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. J. Appl. Ecol. 41:888–896.
Roché, Jr. B. F., C. T. Roché, and R. C. Chapman. 1994. Impacts of grassland habitat on yellow starthistle (*Centaurea solstitialis*) invasion. Northwest Sci. 68:86–96.
Roché, Jr. B. F. and C. J. Talbott. 1986. The collection history of *Centaurea*'s found in Washington state. Pullman, WA: Washington State University Cooperative Extension, Washington State University, Agricultural Research Center Bulletin XB0978. 36 p.
Roché, C. T. and D. C. Thill. 2001. Biology of common crupina and yellow starthistle, two Mediterranean winter annual invaders in western North America. Weed Sci. 49:439–447.
Roché, C. T. and G. R. White. 2002. Managing yellow starthistle in southwestern Oregon. Bulletin EM 8750. Corvallis, OR: Oregon State University Extension, Oregon State University. 8 p.
Sakai, A. K., F. W. Allendorf, and J. S. Holt, et al. 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32:305–332.
Sheley, R. L., L. L. Larson, and J. S. Jacobs. 1999. Yellow starthistle. Pages 408–416 *in* R. L. Sheley and J. K. Petroff, eds. Biology and Management of Noxious Rangelands Weeds. Corvallis, OR: Oregon State University Press.
Sheley, R. L., L. L. Larson, and D. E. Johnson. 1993. Germination and root dynamics of range weeds and forage species. Weed Technol. 7:234–237.
Siemann, E. and W. E. Rogers. 2001. Genetic differences in growth of an invasive tree species. Ecol. Lett. 4:514–518.
Stanton, M. L. 1984. Seed variation in wild radish: effects of seed size on components of seedling and adult fitness. Ecology 65:1105–1112.
Sun, M. 1997. Population genetic structure of yellow starthistle (*Centaurea solstitialis*), a colonizing weed in the western United States. Can. J. Botany 75:1470–1478.
Thébaud, C. A. and D. Simberloff. 2001. Are plants really larger in their introduced ranges? Am. Nat. 157:231–236.
Uygur, S., L. Smith, F. N. Uygur, M. Cristofaro, and J. Balciunas. 2004. Population densities of yellow starthistle (*Centaurea solstitialis*) in Turkey. Weed Sci. 52:746–753.
van der Meijden, E. 1996. Plant defense, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. Entomol. Exp. Appl. 80:307–310.
Wagenitz, G. 1975. *Centaurea* L. Pages 465–585 *in* P. H. Davis, ed. Flora of Turkey, Volume 5. Edinburgh: Edinburgh University Press.
Williamson, M. 1993. Invaders, weeds, and the risk from genetically manipulated organisms. Experientia 49:219–224.
Willis, A. J. and B. Blossey. 1999. Benign climates don't explain the increased vigor of non-indigenous plants: a cross continental transplant experiment. Biocontrol Sci. Technol. 9:567–577.
Willis, A. J., J. Memmott, and R. I. Forrester. 2000. Is there evidence for the post-invasion evolution of increased size among invasive plant species? Ecol. Lett. 3:275–283.
Wulff, R. 1986. Seed size variation in *Desmodium paniculatum*, III: effects on reproductive yield and competitive ability. J. Ecol. 74:115–121.
Wulff, R. D., H. F. Causin, O. Benitez O, and P. A. Bacalini. 1999. Intraspecific variability and maternal effects in the response to nutrient addition in *Chenopodium album*. Can. J. Botany 77:1150–1158.

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